Standard Methods for the Bacterial Examination of Milk

Standard Methods for the Bacterial Examination of Air

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By Committees of the
Laboratory Section
American Public
Health Association

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REPORT OF THE COMMITTEE ON STANDARD METHODS OF BACTERIAL MILK ANALYSIS.

The Committee on Bacterial Milk Analysis respectfully submits the following report for the consideration of the Laboratory Section of the American Public Health Association.

I.

Historical.

At the meeting of the Laboratory Section of the American Public Health Association in Boston in 1905, at the suggestion of Prof. S. C. Prescott, of the Massachusetts Institute of Technology, a Committee was appointed to study the various methods used for the bacteriological examination of milk and to recommend a uniform procedure. This committee consisted of Prof. S. C. Prescott, Chairman; Dr. W. H. Park, Dr. F H. Slack, Prof. H. L. Russell, Prof. C. E. Marshall, Prof. H. C. Harrison and Dr. E. C. Levy.

A circular letter asking for information as to existing methods and technique used in bacteriological milk examinations was sent to leading bacteriologists of the United States and Canada. Many of these did not make such examinations, and the replies of others who did, showed wide variations of procedure on most important points, such as plating, composition of media, incubation temperature, length of incubation, etc.

At the Mexico City meeting in 1906, Prof. H. L. Russell, of the University of Wisconsin, was appointed Chairman of the Committee, but in June, 1907, he asked to be relieved of the chairmanship, and by the vote of the Council of the Laboratory Section, Dr. F. H. Slack, of the Boston Board of Health Laboratory, was appointed Chairman.

At the Atlantic City meeting in 1907 the Committee presented a preliminary statement[1], going into the subject matter in considerable detail. Dr. E. C. Levy withdrew as a member of the Committee and Dr. B. H. Stone was appointed in his stead, otherwise the Committee was continued unchanged.

At the Manitoba meeting in 1908 a report of progress was submitted[2] covering some points on which no recommendations were made in the preliminary statement; the Committee was continued without change. The two preliminary reports of the Committee have been favorably received and the technique recommended has been generally adopted. This final report is practically but a restatement of methods hitherto tentatively recommended.

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Signed, F. H. Slack, Chairman.
W. H. Park, C. E. Marshall,
B. H. Stone, F. C. Harrison,
H. L. Russell.

II.

Collection of Samples. Technique and Apparatus.

Quantities of Milk Required for Analysis. The minimum quantity of milk necessary for making the ordinary bacteriological examination is ten cubic centimeters. When making examinations for certified milk, if possible a pint or quart bottle should be taken and brought to the laboratory unopened.

Collecting Apparatus. In collecting samples for bacteriological examination it is essential that the sample be taken and kept in such a manner as to prevent either any addition of bacteria from without or multiplication of the bacteria originally present.[3] Bottles, tubes, pipettes, etc. used in the collection of samples, besides being washed, shall be sterilized with dry heat for an hour at about 150° C., or to the charring point of cotton.

In the selection of "certified milk" samples it is recommended wherever possible that an unopened bottle be taken, placed in a suitably iced case and brought at once to the laboratory.

Samples of "market milk" may be collected in the same manner as water samples, in sterile, wide-mouthed, glass-stoppered four ounce bottles; the case in which they are carried being well iced. The principal difficulty encountered in this method is in transferring the sample from the original container to the bottle, and the various string and wire devices by means of which the bottle is immersed in the original container are objectionable both on account of the labor of preparing such an outfit and also on account of the coating of milk left on the outside of the bottle when the sample has been taken.

An apparatus designed for the use of test tubes as containers is recommended as superior to one designed for bottles.[4]

Such a case for carrying the samples may be made of copper with double walls, interlaid with half inch felting, outside measure 19 x 9 x 5 inches, inside 18 x 8 x 4 inches, divided into three compartments, the central one 6 x 8 x 4 inches for the samples, the others each 5 x 8 x 4 inches for ice. When iced and closed a constant temperature of 34° to 36° F. is maintained; salt should not be used with the ice or the samples will be frozen. A layer of absorbent cotton will protect the ice from the air when the box is opened for a few minutes. Bits of this cotton are also useful when taking temperatures for quickly wiping the adhering milk from the thermometer.

The samples are carried in cotton-stoppered test tubes 6 x ¾ inches, the compartment holding eight racks of four tubes each. Holes in the bottom of the partitions allow the water to circulate freely about the lower ends of the tubes.

The test tube racks are made of copper tubing weighted with a strip of lead and padded with rubber. When collecting or plating but one rack or a single tube should be removed at a time and the box closed so the other samples will not be exposed to the outside temperature.

Sterile, straight sided, glass pipettes 18 x 3-8 inches with blunt pipette openings 1-8 inch in diameter at the lower ends are used in transferring the samples from the bottles, cans or coolers to the test tubes; these are carried under the sample case in a detachable copper case 19 x 4 x 3½ inches, adapted for sterilizing and divided into two compartments, the upper one for clean sterile pipettes, the lower for pipettes after use, a sterile pipette being used for each sample.

The use of the test tube for the collection of milk samples is recommended instead of bottles for the following reasons, dependent in most cases on the long, slender shape of the tube.

1. Economy of floor area in the collecting case.
2. The facility for maintaining low temperature by the circulation of the ice water about the lower ends of the tubes, thus giving uniformity in the treatment of the specimens.
3. The case with which all the usual washing, sterilizing and general handling of test tubes can be done, since the test tube is a regular piece of apparatus involving no departure from the routine in all the ordinary manipulations.

With samples kept properly iced in this particular form of case there is practically no change in the bacterial content even for 24 hours, the counts varying hardly more than might be expected in duplicate plates. It is recommended, however, that examination of the samples be proceeded with as quickly as possible after the collections are made.

Identification of Samples. When bottles are used identification numbers should be etched on both bottle and stopper. Test tubes should be labelled or etched and numbered.

A complete record of the samples taken, giving date, time, place, name of party from whom sample is taken, name of collector, temperature of milk, character of original container, (tank, can, bottle), etc., should be written opposite duplicate numbers in a blank book or pocket card catalog, or this informa-

tion may be written on small tags and tied or wired to the corresponding test tube or bottle.

TEMPERATURE. The temperature should be taken immediately AFTER taking the sample for analysis, while the milk is still thoroughly mixed.

If it is desired to take the temperature of "certified milk" this should be done when the sample is taken but from another bottle.

A floating thermometer graduated to the Fahrenheit scale is most convenient and the temperature should be expressed to the nearest degree. It is necessary to standardize the thermometer for at least ten degrees on each side of the legal temperature limit. A quickly registering thermometer should be left for at least one minute in the milk and read as soon as removed.

Representative Samples.—Care should be taken to secure a sample which is truly representative of the milk to be examined.

One of several methods for mixing the milk may be used, comparison having shown the results to be practically the same.[3]

1. Pouring the milk into a sterile receptacle and back.

2. Shaking the milk thoroughly with receptacle turned upside down (this may be done where the can or bottle is tightly stoppered or capped and is not so full as to prevent thorough agitation.)

3. In open tanks in stores it is allowable to stir thoroughly with the long handled dipper generally found in use.

4. Where the test tube collecting case is used thoroughly reliable results are secured by first shaking the can or bottle and then stirring with the large pipette before taking the sample, care being taken to close the upper end of the pipette with the finger so that no milk enters until after mixing, or the pipette may be emptied after stirring before the sample is taken.

5. For certified milk samples it is recommended that on arrival at the laboratory the bottle be opened with aseptic precautions and the milk thoroughly mixed by pouring back and forth between the original bottle and a sterile bottle. Another method is to mix as thoroughly as possible by agitation for two minutes in the original container before opening same.

The interval between collection and analysis.—Generally speaking the shorter the time between collection and examination of milk samples the more accurate will be the results. For

routine work the attempt should be made to plate within four hours of the time of collection.

Too much stress cannot be laid on keeping the samples properly iced during this interval. They should be kept below 40° F., but care should be taken that they are not frozen.

III.

MEDIA. Method of making.[5]

AGAR. The standard medium for determining the number of bacteria in milk shall be agar 1%, reaction +1.5, Fuller's scale, made as follows:

1. Boil 10 grams of thread agar in 500 cc. of water for half an hour and make up weight to 500 g. or digest for 10 minutes in the autoclave at 110° C. Let this cool to about 60° C.
2. Infuse 500 g. finely chopped lean beef for twenty-four hours with its own weight of distilled water in the refrigerator.
3. Make up any loss by evaporation.
4. Strain infusion through cotton flannel, using pressure.
5. Weigh filtered infusion.
6. Add Witte's peptone 2%.
7. Warm on water bath, stirring until peptone is dissolved and not allowing the temperature to rise above 60° C.
8. To the 500 grams of meat infusion (with peptone) add 500 grams of the 2 per cent. agar keeping the temperature below 60° C.
9. Heat over boiling water (or steam) bath thirty minutes.
10. Restore weight lost by evaporation.
11. Titrate after boiling one minute to expel carbonic acid.
12. Adjust reaction to final point desired +1.5 by adding normal sodium hydrate.
13. Boil two minutes over free flame constantly stirring.
14. Restore weight lost by evaporation.
15. Filter through absorbent cotton or coarse filter paper, passing the filtrate through the filter repeatedly until clear.
16. Titrate and record the final reaction.
17. Tube (10 c. c. to a tube) and sterilize in autoclave 1 hour at 15 lbs. pressure or in the streaming steam for twenty minutes on three successive days.

All variations from agar media made as described shall be considered as special media. The above medium is recommended as giving the highest and most uniform counts so far as our comparative work has extended. Methods by which the other media mentioned in this report were made are as follows:

BOUILLON. Infuse 500 g. finely chopped lean meat 24 hours with 1,000 c. c. distilled water in refrigerator. Restore loss by evaporation. Strain infusion through cotton flannel.

Add 1% peptone. Warm on water bath, stirring until peptone is dissolved.

Heat over boiling water, or steam bath thirty minutes. Restore loss by evaporation.

Titrate, adjust reaction to +1% by adding normal sodium hydrate.

Boil two minutes over a free flame, constantly stirring. Restore loss by evaporation.

Filter through absorbent cotton, passing the liquid through until clear. Titrate and record final reaction. Tube, using 10 c. c. to each tube. Sterilize.

AESCULIN BILE SALT MEDIUM. (LIQUID).[6]

Weigh out.
1 or 2% of Witte's peptone.
.5% Sodium taurochlorate (commercial).
.1% aesculin.
.05% Ferric citrate.
100 c. c. tap water.

After steaming 15 to 30 minutes the medium is filtered and filled into test tubes and sterilized (fractional sterilization).

PREPARATION OF AESCULIN BILE SALT AGAR.[6]

The directions for making a liter of aesculin bile salt agar are as follows: Boil until dissolved 15 grams of agar, 2.5 grams commercial bile salt, and 10 grams peptone (Witte) in 1,000 c. c. of distilled water. Neutralize with a normal solution of sodium hydrate. Cool below 60° C., add the whites of two eggs or a sufficient quantity of a solution of albumen, bring to the boil and filter as soon as the albumen has coagulated properly. Try the acidity and neutralize if necessary, and then add to the clear

hot filtrate—1 gram aesculin (Merck) and 1 gram iron citrate scales (Merck). After these substances are dissolved test the acidity with decinormal soda solution. It will be found to be about +0.6, as a solution of 1 gram iron citrate scales in 1,000 c. c. water gives an acidity of +0.56. In case the acidity is too high add alkali until the reaction is +0.6, and if the acidity is too low add more iron citrate until the reaction is +0.6. By following these directions exactly satisfactory and even results will be obtained. We have to emphasize here the different manner of neutralization from that recommended for ordinary media by the Committee on Standard Methods of the American Public Health Association, as the procedure outlined above is absolutely necessary. The main point of our reaction is the forming of the black colored salt in sufficient quantity to form as easily visible field.

Lactose (or Dextrose) Litmus Agar, made as ordinary agar with the addition of 1% lactose (or dextrose) to the medium just before sterilization. Reaction shall be made neutral to phenolphthalein. If the medium is to be used in tubes the sterilized azolitmin solution shall not be added until just before the final sterilization. If the medium is to be used in Petri dishes the sterilized azolitmin shall not be added to the medium until it is ready to be poured into the dishes.

Whey Agar. A liter of fresh skimmed milk at 41° C. is loppered by adding sufficient rennet (about 1 c. c. of liquid rennet in 20 c. c. of distilled water). After the curd is firm it is cut in fine pieces and placed in steam for forty minutes. It is then strained through muslin to remove the curd. The reaction of the whey is adjusted to +1.5 acid with the standardized NAOH and 1% of dry peptone and 1.5% of finely shredded agar is added. It is then placed in the steam for 1 hour. The acidity is readjusted to +1.5%. It is then cooled to 60° C. and clarified with egg. Counterpoised and boiled over a free flame for 5 minutes. Filtered through cotton or a hot, washed plaited filter paper, tubed, sterilized 15 minutes for 3 successive days in steam.

Commercial bile-salt may be obtained from Baird & Tatlock, Cross Street, Hatton Garden, London, England, costing about $2.50 per lb.

IV.

Plating. Apparatus. Technique.

Plating apparatus—For plating it is best to have a water bath in which to melt the media and a water jacketed water bath for keeping it at the required temperature; a wire rack which should fit both the water baths for holding the media tubes; a thermometer for recording the temperature of the water in the water jacketed bath, sterile one c. c. pipettes, sterile petri dishes, and sterile dilution water in measured quantities.

Dilutions—Ordinary potable water, sterilized, may be used for dilutions. Occasionally spore forms are found in such water which resist ordinary autoclave sterilization; in such cases distilled water may be used or the autoclave pressure increased. With dilution water in eight-ounce bottles calibrated for ninety-nine cubic centimeters and in test tubes calibrated for nine cubic centimeters, all the necessary dilutions may be made.

Short, wide-mouthed "Blakes" or wide mouthed French square bottles are more easily handled and more economical of space than other forms of bottles or flasks.

Eight ounce bottles are the best, as the required amount of dilution water only about half fills them, leaving room for shaking. Long-fibre, non-absorbent cotton should be used for plugs. It is well to use care in selecting cotton for this purpose to avoid short fibre or "dusty" cotton, which gives a cloud of lint-like particles on shaking. Bottles and tubes should be filled a little over the 99 c. c. and 9 c. c. marks to allow for loss during sterilization.

The dilutions recommended are 1-10, 1-100, 1-1,000, 1-10,000, 1-100,000 and 1-1,000,000.

For certified milk the 1-100 dilution should be used, while 1-100 and 1-10,000 will usually be found best for market milk.

The 1-10 dilution is prepared by shaking the milk sample twenty-five times and then transferring 1 c. c. of the milk to a test tube containing 9 c. c. of sterile water.

The 1-100 dilution is prepared in the same way, except that a bottle with 99 c. c. of sterile water is substituted for the test tube.

The 1-1,000 dilution is prepared by first making the 1-100 dilution, shaking twenty-five times and transferring 1 c. c. of the dilution to a test tube containing 9 c. c. of sterile water.

The 1-10,000, 1-100,000 and 1-1,000,000 dilutions are made in the same manner by dilutions of 1-100, 1-1,000 and 1-10,000 dilutions, 1 c. c. to 99 c. c. of sterile water.

It is recommended that that dilution be used which will produce about 200[7] colonies to a plate, ranging from 40 to 200; where a 1-10 dilution exceeds this number the 1-100 dilution is more accurate, etc. The number of bacteria present, may, if desired, be approximately estimated before dilutions are made by direct microscopic examination of a properly prepared sediment. Otherwise, it is necessary to make a range of dilutions, thereafter selecting for record the count obtained on that plate which yields between 40 and 200 colonies.

Plating whole milk is unreliable, whatever quantities be used, since the bacteria are not so well separated as in the dilutions, and often, owing to the crowded conditions, only a portion of the bacteria present will develop into visible colonies. Moreover if a cubic centimeter of the milk is used, the turbidity of the jelly due to the presence of the milk hides the colonies present from the eye.

Porous earthenware Petri dish covers are recommended as superior to glass since they absorb the excess moisture.[8] They also have the advantage of being cheaper and more durable than glass; they are easily marked with ordinary lead pencil. With long incubation a tendency of plates with these covers to dry out has been observed by some workers; for ordinary routine work however they are perfectly satisfactory using 10 c. c. of media to the plate and incubating in a saturated atmosphere. These covers should never be washed but always thoroughly dry sterilized before use.

Another method of preventing spreaders is by inverting the dishes and placing in the glass cover of each a strip of sterile filterpaper moistened with one large drop of glycerine. Plates so treated do not dry out as quickly as with the porous tops and the glass ware does not become scratched.

PIPETTES. Straight sides 1 c. c. pipettes are more easily handled than those with bulbs; they may be made from ordinary 3-16 inch glass tubing and should be about 10 inches in length.

PLATING TECHNIQUE.[9] The agar after melting should be kept in the water jacketed water bath between 40° C. and 45° C. for at least fifteen minutes before using to make sure that the agar itself has reached the temperature of the surrounding water. If used too warm the heat may destroy some of the bacteria or retard their growth.

For routine work in cities in order to bring down the actual number of colonies in a plate to about the standard of two hundred, it is well to use a dilution of 1-10,000. To make this dilution use two bottles of sterile water each containing 99 c. c.

Shake the milk sample twenty-five times, then with a sterile pipette transfer 1 c. c. to the first dilution water and rinse the pipette by drawing dilution water to the mark and expelling; this gives a dilution 1 to 100.

Shake the first dilution twenty-five times, then with a fresh sterile pipette transfer 1 c. c. to the second dilution water, rinsing the pipette to the mark as before; this gives a dilution 1-10,000. Shake the second dilution twenty-five times, then with a sterile pipette transfer 1 c. c. to the Petri dish, using care to raise the cover only as far as necessary to insert the end of the pipette.

Take a tube of agar from the water bath, wipe the water from outside the tube with a piece of cloth, remove the plug, pass the mouth of the tube through a flame, and pour the agar into the plate, using the same care as before to avoid exposure of the plate contents to the air.

Carefully and thoroughly mix the agar and diluted milk in the Petri dish by a rotary motion, avoiding the formation of air bubbles or slopping the agar, and after allowing the agar to harden for at least fifteen minutes at room temperature place the dish bottom down in the incubator.

CONTROLS. Plating should always be checked by controls. A blank plate should be made with each series of milk plates for control on the agar, water, air, Petri dishes, pipettes, etc.

For control of the technique of plating, it is recommended that for work on "market milk" duplicate plates be made each day on several samples.

"Certified milk" should always be plated in duplicate and where it is possible it is well to have one man's work occasionally checked by another.

Unless duplicate plates show as a rule approximately the same count the worker should see if there is error in his technique.

Plating should always be done in a place free from dust or currents of air.

In order that colonies may have sufficient food for proper development 10 c. c. of agar shall be used for each plate. In plating a large number of samples at one time, the dilution and transfer of diluted milk to the plates may be done for four or eight samples, then the agar poured, one tube to each plate, then another eight samples diluted, etc.

V.

Incubation and Counting.

Two standard temperatures are recognized.

1. 48 hour incubation at 37° C.
2. Five day incubation at 21° C.

Regulations governing the number of bacteria allowable in milk should direct the method to be used in examination and in all reports, papers, etc., on the bacterial count of milk this factor should be explicitly stated.

Incubators should be carefully regulated. Whatever temperature of incubation may be used it is important that the incubator air should be saturated with moisture; this may be accomplished by either having a depression in the floor of the incubator filled with water or by setting a pan of water on one of the shelves.

Counting. Expression of results. Since minor differences in milk counts are within the working error of the methods and are of no significance in practice, the following scale has been adopted for recording results of market milk examination.

Counts below 50,000 are distinguished by five thousands.

Counts between 50,000 and 100,000 are distinguished by ten thousands.

Counts between 100,000 and 500,000 are distinguished by fifty thousands.

Counts between 500,000 and 5,000,000 are distinguished by hundred thousands.

Counts above 5,000,000 are distinguished by millions.

Therefore only the following figures are used in reporting.

5,000	400,000
10,000	450,000
15,000, etc., to 50,000	500,000
60,000	600,000
70,000	700,000
80,000	800,000
90,000	900,000
100,000	1,000,000
150,000	1,100,000, etc., to 5,000,000
200,000	6,000,000
250,000	7,000,000
300,000	8,000,000, etc., by millions.
350,000	

Counts on "certified" or "inspected" milk shall be expressed as closely as the dilution factor will allow.

The whole number of colonies on the plate shall be counted, the practice of counting a fractional part being resorted to only in case of necessity, such as partial spreading.

Various counting devices have been recommended by different workers. The more simple ones, where the whole plate can be seen at once, are more desirable on account of there being less likelihood of recounting colonies. Colonies too small to be seen with the naked eye or with slight magnification shall not be considered in the count.

VI.

Milk Sediments.

It is probable that within the past five years more research work has been done in relation to the various sediment or centrifuge tests for milk than any other method of examination.

These tests were originally started with the idea of detecting mammitis by noting the increase in the polynuclear cells and have become amplified to such an extent that excepting for certified milk a fairly satisfactory bacterial analysis of milk may be made by these methods alone. Roughly these tests now include (a) Estimation of leucocytes, (b) Estimation of number of bacteria together with morphology of same, (c) Estimation of

foreign matter, dirt, feces, etc. It is however impossible to group them separately under these headings since by some of the methods all of these determinations are made. For the most accurate leucocyte counting the Doane-Buckley test as modified by Russell and Hoffman is recommended, while the Stewart method as modified by Hill and Slack is recommended for routine inspection work.

Leucocytes are present in all normal milks and their number occasionally fluctuates greatly without apparent cause. Milk from animals suffering from udder inflammations almost constantly shows a high leucocytic content and without question is unfit for human consumption.[10]

While there is no point in the milk from a single animal where we can say it passes from normal to abnormal in this respect, enough research has been made to prove that the mixed milk from several normal animals very seldom exceeds 500,000 leucocytes to the cubic centimeter. While healthy cows with no distinguishable lesions may occasionally for short periods pass this limit, such variations are very transient in character and if the mixed milk from several cows shows such high content of leucocytes it raises a suspicion of some abnormal condition.[11]

While a leucocytic count of 500,000 or more to the cubic centimeter in the case of a single animal may be transient and negligible, when found in mixed milk it is sufficient evidence to warrant the exclusion of such milk from the market, until satisfactory veterinary inspection of the herd is made.

Stokes[12] devised a microscopic examination of milk for pus cells and streptococci as a means of detecting the presence of mammitis among cows supplying the milk. Centrifugal sediment from ten cubic centimeters of milk was stained and examined with one-twelfth oil immersion lens. He regarded the presence in the milk of an individual cow of five cells per field of the oil immersion lens as justification for excluding the animal from the herd.

Bergey[13] modified Stoke's method and made extensive examinations of the milk of individual cows. Parallel bacteriological examinations of the milk for both species and numbers supplemented his examinations for cells.

Tromsdorff[14] devised a method which consists in centrifugalizing 5 c. c. of milk in a special centrifuge tube with a lower constricted portion so graduated as to permit of reading off directly the amount of sediment. The mixed milk of cows with sound udders, as a rule, shows sediment varying from traces to .5 c. c. per liter with 1 c. c. per liter as the maximum. Trommsdorff recommends the test as an aid in the detection of chronic mammitis.

Stewart[15] of the Philadelphia Bureau of Health further modified Stoke's method so that it was practicable to use it for the examination of large numbers of samples of mixed herd milk. Stewart describes the apparatus and method as follows:

"This apparatus consists of a circular pan about 12 inches in diameter and ¾ inches deep, containing twenty small glass tubes. The tubes contain 1 c. c. of milk and are filled by means of a small bulb similar to that ordinarily used on medicine droppers. The end of the tube is closed by a small rubber stopper, and the tubes are held in the pan by clamps. This pan is fitted upon the ordinary Beckel water centrifuge and covered with a lid which is held down by a thumb screw. The pan covered in this way furnished a surface of very slight resistance to the atmosphere during its revolution, somewhat on the principle of a child's top."

By the old method the arms of centrifuge containing the milk encountered so much resistance in their revolution that the speed with 15 lbs. water pressure was not more than 1,200 revolutions per minute, while the speed obtained with the new apparatus is from 2,500 to 3,000 revolutions per minute with 15 lbs. pressure. This rapid speed causes sedimentation to occur in less than five minutes. When this is completed the centrifuge pan can be lifted from the motor and the per cent. of cream measured by a graduated scale marked upon the tube. The heavier matter, as the insoluble dirt, pus cells and bacteria, is thrown to the peripheral end of the tub where it adheres to the rubber cork in the lumen of the tube. To examine this sediment the cork is carefully removed and a spread made by rubbing the cork containing the sediment over an area of a square centimeter on a 3-inch by 6-inch glass slide. The proper area of the smear is obtained by placing underneath the slide a scale of circles having an area of

a square centimeter. After the smears are dried in the air without fixation of heat, the preparation is stained by the Jenner blood stain for two minutes, keeping the stain in constant motion. The excess of stain is washed off in water and the preparation is dried in the air. By this blood staining method the pus and blood cells are stained perfectly and the ordinary micro-organisms take the blue stain well.

The stained specimens are examined with a one-twelfth oil immersion objective and a No. 3 eye piece. The character of the bacteria is noted and the average number of pus cells per field is counted. This average number is multiplied by 4,400 since there are about 4,400 fields to a square centimeter as estimated by the stage micrometer. This result is approximately the number of pus cells per cubic centimeter.

Hill & Slack[16] modified the Stewart method by using tubes of a larger bore containing two cubic centimeters, stoppered at each end, centrifugalizing at a speed of 2500 revolutions a minute for 10 minutes and smearing the sediment evenly over 4 square centimeters with a drop of sterile water. The advantages claimed over the Stewart method are:

The tubes are so large that there is practically no capillary action. The time of centrifugalizing is increased. The use of water with the smear allows of a thin even smear, the small amount of material of the same dilution left on the stopper being negligible for all practical purposes.

The number of leucocytes per 1-12 oil immersion field multiplied by 20,000 gives the approximate number per cubic centimeter.

So little of the sediment is seen at once with the high power that it is well to confirm the diagnosis of pus by making a thorough examination of the whole surface with a low power lens, to determine how uniform a smear has been made. By using an eye-piece micrometer ruled in squares, the relation of one square to that of 1-12 immersion lens being previously calculated, a count may be made with the low power lens.

The Doane-Buckley[17] quantitative method of estimating leucocytes is described by them as follows:

"With this method ten cubic centimeters of milk are centrifuged for four minutes in graduated sedimentation tubes, at an approximate speed of 2,000 revolutions per minute. The

cream is lifted out with a cotton swab, care being taken to get as much as possible of the fat. It is then centrifuged one minute more and the cream again removed with a cotton swab. Any fat remaining in the milk interferes seriously with the counting, as, if there are more than a few globules they form a layer on the top of the liquid in the counting chamber, and as the leucocytes settle to the bottom of the chamber, it is difficult to see through the fat. It is only with cows giving milk difficult of separation where this trouble is experienced, and with such animals considerable care is necessary in removing all the cream gathered at the top of the sedimentation tube. The method of removing the fat with cotton is the best one that has occurred to us, and it is the only part of the process that does not operate with entire satisfaction in every instance.

Following the removal of the cream, after the second centrifuging the bottom of the tube will contain a portion of the sediment which is easily seen. This sediment may, in extreme cases of cows suffering from garget amount to as much as one cubic centimeter. Ordinarily it will be considerably less than one-half cubic centimeter. The amount varies considerably with the number of leucocytes, but not absolutely. The milk above this sediment is removed with a small siphon, which can be easily arranged with bent glass tubes drawn to a fine point and supplied with a small rubber end pinch cock. In using the siphon it is better to keep the point near the surface of the milk in the tube in order not to agitate the precipitated leucocytes and draw a number of them off with the milk. The milk in the tube may be siphoned within an eighth of an inch of the sediment in the tube. This will usually be below the ½ c. c. mark. Two drops of saturated alcoholic solution of methylene blue are then added, thoroughly mixed with the sediment by shaking, and then set in boiling water for two or three minutes to assist the leucocytes in taking the color. The contents of the tube can be boiled by holding it directly in the flame, but it has no advantage over the use of the water bath, and it is very likely to break the glass. After heating, some water is added to the tube to render the color less dense. Ordinarily filling the tube to the 1 c. c. mark will be sufficient, and this quantity gives an easy factor for calculating the final results.

In putting this liquid containing the leucocytes into the blood counter considerable care is necessary, owing to the tendency of the leucocytes to sink to the bottom. At this place a capillary tube is used, and the cover glass was held in one hand ready to cover the chamber as soon as the drop was transferred to the counting counter. After placing the glass cover over the chamber, about a minute is allowed the leucocytes to settle to the bottom of the chamber. There are very few foreign bodies likely to be mistaken in counting for leucocytes. Ordinarily the polynuclear leucocytes predominate and the stained nuclei with the unstained surrounding cell show up very distinctly. A few small leucocytes with large nuclei may be found and these may be confounded with yeast cells until the worker becomes familiar with the distinction.

As regards counting we have taken a standard with a cubic centimeter as a basis quantity of milk, though we are of course aware that the corpuscles in the blood are enumerated with a cubic millimeter basis. We adopted the centimeter largely for two reasons. In counting bacteria in the milk the cubic centimeter is always the basis employed. Simply because the leucocytes were derived from the blood seemed to be no reason why the same basis for counting should be employed as was used with the blood, while to the ordinary bacteriological worker to whom this work will fall, if ever adopted to any extent, the cubic centimeter standard would be a little more easily comprehended because more frequently used. The blood counter holds one-tenth cubic millimeter and one-ten-thousandth cubic centimeter. If ten cubic centimeters of milk are used and the 1 cubic centimeter of fluid is in the tube after siphoning, and the coloring matter and the water used to dilute has been added, then the resulting number of leucocytes in the counting multiplied by 1,000 will be the total number of leucocytes per cubic centimeter in the milk. If a total of 75 leucocytes was counted in the chamber there would be 75,000 leucocytes per cubic centimeter in the milk.

In the actual counting under the microscope a square millimeter of the counting chamber will be found to be ruled off into 400 smaller equal squares. This facilitates an accurate and rapid count. Where the number of leucocytes is not great the

entire field can be counted in a short time. Where there is a great number of leucocytes a few squares or sets of squares in different parts of the ruled surface will give approximately the number.

There are occasionally a few variations desirable from these rules, but it may be well to state that the details have been pretty carefully and thoroughly worked over and compared, and it is seldom that short cuts can be made if correct results are desired. The time and speed of centrifuging are placed as low as possible for accurate work. When there is one-half c. c. or more of sediment, it is necessary to use more of the methylene blue for staining, as there will be too great a number of leucocytes to make a satisfactory count in the counting chamber, it is better to add water until there are two cubic centimeters, or sometimes even more in the sedimentation tube.

This method of counting, while long in explaining is in reality short and simple in application. Moreover, it is based on accurate measurements in every detail, and the results are correspondingly reliable."

Savage[18] devised a method similar to that of Doane & Buckley which, like theirs, shows large numbers of leucocytes in the milk of normal cows.

Russell & Hoffman[19] working farther with the Doane & Buckley method have made several modifications and recommend the following procedure:

Collection of Samples. Samples for analysis should be taken from the entire milking of the animal, as the strippings contain a somewhat larger number of cells than other portions of the milk. For the purpose of examination take 200 c. c. in stoppered bottle.

Time Interval between Collection and Analysis. To secure satisfactory results, milk must be examined in a sweet condition. Development of acidity tends to precipitate casein in the milk and thus obscure the examination of microscopic preparations. Samples received from a distance can be preserved for satisfactory microscopical examination by the addition of formalin at the time of collection—a proportion of 1 c. c. to 250 c. c. of milk. Formalin has been found the best preservative to use although it causes contraction of the cells to some extent.

PROCEDURE WITH REFERENCE TO PREPARATION OF SAMPLE.

1. Heating sample.—To secure the complete sedimentation of the cellular elements in the milk, it is necessary to heat the same to a temperature which will break down the fat globule clusters, or lessen the ordinary creaming properties of the milk. Samples should be heated at 65° to 70° C. for not less than ten minutes, or from 80° to 85° where very short periods of exposure (one minute) are given. This treatment causes the more homogenous distribution of the fat globules through the milk, and when the sample is then subjected to centrifugal force, the cell elements are not caught in the rising fat globules, but on account of their higher specific gravity are concentrated in the sediment by centrifugal force.

2. Concentration of cellular elements—Ten c. c. of milk are placed in an ordinary sedimentation tube, and after heating as above directed and subsequently shaking, the milk is centrifugalized twenty minutes at 1,200 revolutions per minute. A hand centrifuge may be employed for this purpose; where available a steam turbine Babcock milk tester may be found more practicable.

NOTE.—This speed maintained for the time mentioned, is sufficient to sediment practically all the cell elements suspended in the milk. In our experience we have found the number of cells in supernatant milk to average only 3½%.

3. Preparing the sample for examination—After centrifugalizing, the cream and the supernatant milk are removed, with the exception of the last ½ c. c., by aspirating with an exhaust pump and wiping the walls of the tube with a cotton swab. After thoroughly mixing the sediment with a glass rod, enough of the emulsion is placed in an ordinary blood counter (Thoma Zeiss pattern) to fill exactly the cell. The preparation is then allowed to stand for a minute or two to permit the cellular elements to settle to the bottom of the cell while the few fat globules in the liquid rise to the surface. This method permits of the differentiation of the cells from the small fat globules, in the liquid rise so that a distinct microscopic observation can be made.

EXAMINATION OF MATERIAL. The preparation is examined in an unstained condition.

NOTE.—Most observers have usually stained the sediment prior to examination, but we have found with the above treatment that the cells may be enumerated quite as well in an unstained condition as a stained condition.

The count is made with a one-inch eye piece and 1-6 objective. Where the number of cell elements exceed 12 or 15 per microscopic field, above referred to, one-fourth of the entire ruled area of the counter, equivalent to 100 of the smallest squares of the cell, are counted. Where the cell elements are less abundant, one-half of the entire area (two to four hundred squares) are enumerated. The average number of cells per smallest square is then obtained, which when multiplied by 200,000 gives the number of cells per cubic centimeter in the original milk; multiplied by four million we have the number of cells per cubic cenitmeter in the sediment examined. As the sediment represents the concentration of cells into one-twentieth of the original volume of milk taken (10 c. c. to one-half c. c.) this number should be divided by twenty to give the number of cells per cubic centimeter in the original milk.

NOTE.—The above factor of 4,000,000 is obtained as follows: The cubic content of the blood counter represents one-tenth of a cubic centimeter. This volume is divided by means of the ruled scale into 400 small cubes each equal to one four-thousandth of a cubic millimeter, or one four-millionth of a cubic centimeter.

EXPRESSION OF RESULTS. All results should be expressed in number of cells per cubic centimeter of the original milk, and in order to avoid fictitious accuracy, should be given in accordance with the method adopted by the Committee on Standard Methods of Water Analysis, as reported in the report in the Laboratory Section of the American Public Health Association for 1905, page 94.

STONE & SPRAGUE have devised a centrifuge tube for quantitative and qualitative analysis of milk sediment which is practically a combination of the Stewart and Trommsdorff tubes, being a 15 c. c. tube, the lower portion of which is drawn into a finely graduated tube about 1-16 inch in diameter and closed with a rubber stopper.

For this method of examination they claim the following advantages:

"First, it measures more accurately the sediment. Second, smears of the sediment can be made in the same manner as with the Slack tube. Third, the column of sediment tends to become stratified so that the different elements can be easily made out. Fourth, the tubes are much more easily cleaned. With this tube we have been able to detect without microscopical examination, the presence of cases of acute mastitis when the milk was diluted thirty times with normal milk. For ordinary routine work we think that centrifugalization in these tubes, using one-tenth cubic centimeter of a leucocyte sediment to fifteen cubic centimeters of milk, in a filled tube, as a maximin limit, will be of as much value as a count. The character as well as the quantity of the sediment should of course be taken into consideration. A sediment from cases of even slight mastitis practically always having a yellowish or pinkish tinge of pus, mixed with a smaller or larger amount of blood. Microscopical examination usually shows red cells as well as pus cells. This test should of course be supplemented by an actual examination of the herds in every case."

The Microscopic Estimate of Bacteria. (Slack).[20] "The apparatus and the method for making the microscopic estimate are as follows: The special apparatus for centrifugalizing the milk, modified from one used for leucocyte estimation by Stewart of Philadelphia consists of an aluminum disk and cover, 10 inches in diameter and 5-8 inch in depth, fitted to hold twenty small glass tubes arranged radially. These tubes hold about 2 c. c. each and are closed at both ends with rubber stoppers.

The milk samples are thoroughly shaken, the tubes filled, stoppered, inserted into their proper numbered receptacles in the disk, and centrifulgalized for ten minutes at a speed of from two to three thousand revolutions per minute. Thus in each tube the whole sediment from a known quantity of milk is obtained, and may be spread over a given area. A space about 4 sq. cm. is most convenient, being the right size to allow thorough emulsion of the sediment with a drop or two of sterile water, and to permit drying into a thin even smear. It is convenient to smear a number of samples consecutively on a long glass slide which has previously been correctly spaced with a blue pencil.

To obtain the sediment with the least disturbance, the stopper is first removed from the inner, or cream end, then the tube is held with the cream end downwards, the cream removed with a platinum loop and the milk poured out; lastly, still holding the cream end down, the other stopper is carefully removed with the adhering sediment and the sediment smeared evenly with a drop of sterile water over the space on the glass slide, the stopper being rubbed directly on the glass until the sediment has been transferred. When this is properly done the amount of diluted sediment remaining on the stopper is practically negligible. The smear is then dried with gentle heat and stained with methylene blue.

The microscopic examination of a milk sediment thus easily prepared reveals more than any other single test. It shows the character of the milk, the approximate number and morphology of the bacteria, and the presence of pus or streptococci.

It is not claimed that all the bacteria in the milk subjected to centrifugalization are precipitated into the sediment; but it is claimed that in 99 per cent. of the samples a representative number, is so precipitated, and that this number bears a fairly constant relation to the 1-10,000 dilution plate culture when grown in a saturated atmosphere at 37° C.* for twenty-four hours, 1 per cent. agar being used with a reaction of +1.5.

We may say as a rough estimate, that each coccus, bacillus, diplococcus, or chain in the 1-12 oil immersion field represents one colony in the 1-10,000 plate from the same sample. In most cases the count of a representative field multiplied by 10,000 gives approximately the number of bacteria per cubic centimeter. By the use of this method a good idea of the condition of a single sample of milk can be obtained in less than twenty minutes. Thirty samples can be examined in an hour. At the contractor's receiving station one can easily examine 100 to 200 samples daily, thus keeping close watch over the dairies.

In ordinary routine city inspection only those samples need be plated which are doubtful or above the limit established. In this work the plate would corroborate the microscopic findings

* The method was originally worked out in relation to the 24 hour count at 37° C. The exact factor would have to be figured in accordance with the area of the 1-12 immersion field of the individual microscope. The value of the test lies, however, not in accurate counting so much as in the decisive picture obtained of the character of the milk.

and strengthen the evidence of the court cases. Where plates are to be made the microscopic estimate gives an indication of the proper dilution to use.

VII.

Special Bacteria and Tests for Same.

Pathogenic Organisms. Typhoid and diphtheria bacilli are rarely sought for in milk because it is recognized that although these diseases are often conveyed in milk, the period of incubation is such that by the time the outbreak on a special route is noticed the contagion has usually disappeared from the the milk.[21] Typhoid may be isolated by the use of Lactose bile medium as recommended by Jackson and Melia.[22]

Tubercle Bacilli in Milk.[23] Collection of samples and technique. "Pint or quart samples of milk should be obtained, kept well iced, and delivered to the laboratory as soon as possible. The milk and cream should be well mixed by shaking vigorously. 50 c. c. of the mixed milk are then transferred to a large centrifuge flask and 100 c. c. of sterile water added. Centrifuge for one hour at 2,000 revolutions per minute. The milk was diluted with twice its volume of water with the idea that it would decrease the specific gravity of the milk and so permit of the easier sedimentation of the tubercle bacilli. Guinea pigs are then inoculated, subcutaneously in the belly wall, with 5 c. c. of the sediment thus obtained. The guinea pigs not dying in at least two months are chloroformed, after being tested with tuberculin, and careful autopsies made. Smears, cultures and sections are made from the various organs of the animals that show any change from the normal. The smears are stained with carbol fuchsin and examined for acid fast bacilli.

Cultures are made on glycerinized potato and glycerine agar to rule out Rabinovitch's quick growing acid fast butter bacillus.

Sections are stained with carbol fuchsin for tubercle bacilli and also with haematin and eosin for histological appearances.

Tuberculous guinea pigs may be differentiated from non-tuberculous by giving sufficient crude tuberculin (2 c. c.) subcutaneously to cause the death of the tuberculous animals in twenty-four hours.

Of about 250 guinea pigs tested in this way no animal that did not have tuberculosis died. Two or three that had slight lesions did not die but became sick. It was noted that all the animals died whose lesions had become caseated.

The reaction seems of distinct service in eliminating infections with acid fast organisms and the suggestion is made that with some modification the procedure may have a distinct place as an aid in differentiating true tuberculosis from infections with other acid fast organisms which produce tubercular-like lesions." It is of course understood that the examination of milk for tubercle bacilli is by the very nature of the test limited. For the control of this disease in cattle we must rely upon the tuberculin test.

Wisconsin Curd Test.[24] The Wisconsin curd test is conducted as follows:

1. Sterilize milk containers so as to destroy all bacteria in vessels. This step is very important and can be done by heating cans in boiling water or steam for not less than one-half hour.
2. Place about one pint of milk in a covered jar and heat to about 92° F
3. Add ten drops of commercial extract of rennet and mix thoroughly with the milk to quickly coagulate.
4. After coagulation cut curd fine with case knife to facilitate separation of whey; leave curd in whey one-half hour to an hour, drain off whey at frequent intervals until curd is well matted.
5. Incubate curd at 98° to 100° F. immersing jar in warm water. Keep jars covered to retain odors.
6. After six to nine hours incubation open jars and observe odor, examine curds by cutting with sharp knife and observe.
7. Very bad milk will betray the presence of gas-producing bacteria by the spongy texture of the curd and will have an off-flavor.
8. If more than one sample is tested at the same time, dip knife and thermometer in hot water before each time used."

As a rule milks showing the presence of gas or bad odors in any considerable degree are milks that have been more or less polluted with extraneous organisms or carelessly handled, and as a consequence such milks show a curd filled with pin holes due to

gas. It is not intended that this test should be used for an absolute indication of the presence of gas-producing organisms, but rather it has been of service in the detection of the condition of market milk. It is possible that a milk containing but few bacteria may give a very undesirable curd. In order to obtain a good curd we must either have a milk which contains almost no bacteria or one which contains large numbers of lactic acid organisms. While more valuable in testing milks for cheese making it is useful in the examination of market milk if used with judgment. Work done with this test for the detection of fecel matter shows that positive results can be obtained from other gas formers than *B. coli*. The advantage of the test is that it is simple to perform in the dairies and very quick in its results, determinations being made over night. The disadvantage is that while it is a valuable indicator it is by no means a sure test for fecal matter.

Other methods of detecting gas-producing organisms in milk—Gas producing organisms may be tested for in milk, as in water, with glucose or lactose broth in fermentation tubes. Test similar to presumptive test for *B. coli* in water analysis may be made by inoculating into these broth fermentation tubes a c. c. each of the 1-100, 1-1,000 and 1-10,000 dilutions, or if *B. coli* organisms are to be numerically determined the milk may be plated in lactose litmus agar, red colonies counted and species tests worked out. Lactose-bile medium has also been used for the determination of *B. coli* in milk.

The presence of these gas-producing organisms in abundance usually indicates dirty conditions of stables, cows or vessels. In small quantities they may be found in most milks.

In Baltimore routine examinations are made for *B. coli* in milk in 1-1,000 c. c. of each sample.

One c. c. of the 1-1,000 dilution is placed in ordinary bile containing 1% lactose in a fermentation tube and allowed to stand at 37° for 72 hours, at the end of which time if there is more than 15% gas, plates are made, colonies isolated and run through species tests. Of many hundred examinations the colon bacillus is found in about 25% of the samples in winter and 75% in summer in 1-1,000 of a c. c.

The following table prepared from the results of the routine examination of the Baltimore milk supplies for 1906 shows that the colon bacillus is more apt to be present in milk of high bacterial content.

Number of Bacteria	No. of Samples	*B. coli* present 1-1000 c. c.	*B. coli* absent 1-1000 c. c.
10,000 and under.....	32	15%	85%
50,000 and under.....	92	20%	80%
500,000 and under.....	236	30%	70%
500,000 to 1,000,000.....	64	66%	34%
1,000,000 and over.....	338	72%	28%

Gas Production may be demonstrated by adding to a measured quantity (10 c. c.) of milk in a fermentation tube either 3 c. c. of 5% solution of carbolic acid or 1 c. c. of a sterilized 2% solution of bile salt containing neutral red in sufficient quantity to give the milk a deep pink color. Incubate for 24 hours at 37° The chemicals evidently inhibit the growth of the lactic acid bacteria. Where neutral red is used if the gas producing bacteria are in large numbers the deep pink of the milk is changed to a canary yellow.

Dextrose litmus agar and lactose litmus agar are of use in differentiating acid formers, the former giving better results.

Whey agar favors the growth of lactic acid organisms, but is unfavorable for other types.

In incubation at 21° C. the addition of 1% lactose to agar has given higher counts than agar without.

Determination of Streptococci.[16] "Although by careful searching a few streptococci will be found in most sediments from pus milk they are seldom found to any great extent by direct microscopical examination. Occasionally a sample will be found crowded with long chains; more often, streptococci, if present, are in the form of diplococci or very short chains.

Where streptococci, diplococci or cocci are found in the sediment and the plate from the same sample contains colonies resembling streptococci colonies, these colonies may be grown in bouillon to see if chains will develop.

First make and record an estimate of the number of such colonies present, then transfer from 10 to 50 of them to bouillon and grow for 15–24 hours at 37° C. To examine the bouillon culture, spread a loopful on a glass slide, fix with heat, fix with alcohol while slide is still quite hot, stain with methylene blue, wash immediately, dry and examine.

Streptococci in small numbers are present in most market milks as shown by Heinemann and many of the short chain varieties are undoubtedly at the time harmless, though by passing through animals their pathogenicity may become marked.[25]

Long chain streptococci are more apt to indicate inflammatory reactions[26] and milk containing these in large numbers is certainly not a safe article of diet.

A milk should not be condemned because a few chains are found together with large numbers of other microscopic organisms in a bouillon culture, but it is safer to exclude a milk from the market when these three tests agree.

1. Microscopic examination of the sediment shows streptococci, diplococci or cocci.

2. The plate from the same sample shows colonies resembling streptococci colonies exceeding a count of 100,000 to a cubic centimeter.

3. The bouillon culture from these colonies shows long chain streptococci alone or in great excess compared with the other bacteria present."

Milk showing in the stained sediment both abundance of long chain streptococci and pus should be condemned as unsafe.

VIII.

Laboratory Procedure on Routine Samples.

The following procedure is recommended for routine work.

1. Centrifugalize, make smeared sediment, stain and examine microscopically for approximate number of leucocytes, approximate number of bacteria, types of bacteria, streptococci, etc.

2. Plate at least those samples, as indicated by the microscopical examination which show bacterial content around or more than the number permitted by the regulation.

3. Incubate 48 hours at 37° C. or 5 days at 21° C.

4. Count colonies.

5. From plates showing numerous pin point colonies transfer ten or more to broth and grow 15–24 hours and examine for streptococci.

IX.

Interpretation of Results.[27]

Bacterial Count. A high bacterial count in milk indicates lack of cleanliness in production, or lack of care after production. Age of the milk is also an important factor and in interpreting results the distance milk has to be brought, etc., should be taken into consideration. Thus a count of 100,000 bacteria to a cubic centimeter should be considered a serious contamination in milk which may be delivered to the consumer within a few hours of production, while a count of no higher than 100,000 in milk produced at a distance and say 24 to 36 hours old is evidence of ordinarily good care. To produce a milk averaging under 10,000 bacteria to the cubic centimeter requires the utmost care and watchfulness of each detail.

Leucocytes.—A leucocytic content of 500,000 or over to the cubic centimeter especially in testing mixed milk should be regarded as suggestive of some inflammatory condition of the udder and the milk excluded until after satisfactory veterinary inspection.

Indication of the presence of pus is more sure if the leucocytes are clumped.

Streptococci. Long chained streptococci are sometimes found in the smeared sediment especially in pus milks, their presence in such smears or when found by the plate method in numbers of over 100,000 to the cubic centimeter should be considered sufficient evidence for exclusion of the milk until after satisfactory veterinary examination of the cows.

B. coli are present in most milks, their presence in large numbers in milk should be regarded as evidence of unsatisfactory conditions at the dairy.

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REPORT OF THE COMMITTEE ON STANDARD METHODS FOR THE EXAMINATION OF AIR.

I. Synopsis.

The most important impurities of air, which it is possible to detect and measure in sanitary investigations, are physical, rather than chemical or bacteriological. The evil effects of heat and humidity upon the human organism are universally recognized. Dust particles injure the throat and lungs and play an important part in predisposing to tuberculosis. Bad lighting exerts an obviously harmful effect upon the eyes. Hence the Committee believes that determinations of temperature, humidity, dust and intensity of light should be fundamental in all sanitary investigations. Standard procedures are recommended for all four of these tests.

Chemical determinations of carbon dioxid in the air, while historically of supreme importance, are held by the Committee to furnish less direct evidence of unfavorable hygienic conditions than do the tests for temperature and humidity and dust, (Gilbert, 1909; Great Britain, 1909). In combination with these latter tests they may, however, be of value, and a standard procedure is suggested. In certain special investigations the determination of the number of bacteria present in the air may also be of interest and a standard procedure is recommended for this purpose.

Other minor questions are discussed in the report, without the recommendation of standard procedures.

II. Physical Determinations.

The principal physical properties of air which it is desirable to take into consideration are temperature, humidity, pressure, dust, light and the velocity of air currents. It would be desirable to include sound and odor, but at the present time it seems impracticable to bring these two important properties of air within the range of exact observation and record.

For most practical purposes it is desirable that analyses of air should show average conditions, that is, conditions which

obtain over an appreciable period of time, as, for example, from thirty seconds to several minutes. In most cases the minute changes in the atmosphere which are constantly occurring are of small consequence to the analyst and can be neglected, except in unusually delicate researches, where special apparatus is required. Fortunately most types of physical apparatus intended for the analysis of air are adapted to register these average conditions, so that the records need no calculation to make them suitable for practical use.

The reason why average conditions are recorded is that the instruments have a lag, which makes the reading occur some time after the occurrence of the conditions which produced it. For most purposes this lag or inertia is of little consequence, but in some instruments it is so great as to be seriously objectionable. For example, some types of thermometers take 15 or 20 minutes to record the temperature when a decided change occurs. This lag may make the reading useless, where a thorough knowledge of the changes is important.

1. Temperature.

For most purposes the temperature of the air can most conveniently be determined by means of mercurial thermometers. These are made in a great variety of forms depending upon the uses to which they are to be put. An accurate and convenient form of thermometer is a naked tube with an elongated bulb of mercury at one end and a ring at the other through which a cord can be tied. The scale in degrees and fractions thereof is etched upon the glass. Thermometers of this type may possess considerable accuracy. Generally they can be relied upon to about one-half to one-fifth of one degree.

It is common to place rod thermometers upon a backing of metal, card or wood, the scale in this case not being etched upon the glass but painted upon the backing. It is perhaps unnecessary to say that thermometers of this type are often more ornamental than accurate. They usually possess a decided lag and are, for this reason, frequently unserviceable. When employed for careful air work thermometers should be suspended freely in he atmosphere or, at least, placed in a current of air sufficient to insure good ventilation about the mercury column.

Registering thermometers are of two principal types—those which record maximum and minimum temperatures and those which make a record of all the changes of temperature that occur. The latter instruments are provided with clock works which move sheets of paper under a pen by which the record is made.

The maximum and minimum thermometer is constructed so as to have a small rod of metal free to move in the tube which holds the mercury. A rising or falling column of mercury pushes the metal rod before it, but leaves the rod upon receding again. When necessary the metal rod is brought to the point of contact with the mercury by means of a small horse-shoe magnet manipulated outside of the thermometer tube. Maximum and minimum thermometers of this type are almost invariably mounted upon a backing and consequently have a considerable lag. They are, nevertheless, serviceable where fluctuations in temperature are not rapid and can be recommended for determining the highest and lowest temperatures, under such circumstances.

STANDARD METHOD FOR TEMPERATURE. For an intelligent understanding of the sanitary condition of any room, car or other enclosed space neither single determinations nor maximum and minimum records are sufficient. Recording thermometers should be used, placed at various selected points and records should be obtained covering a period of several days. Such instruments are of several types. Instead of mercury the contracting and expanding medium is some rigid metal or combination of metals whose contraction and expansion causes a pen point to bear over a moving paper scale and so leave an ink trace. The clock work is generally wound up for a week, for which period the paper scale is also adapted. Scales for recording thermometers are of two principal types—those which are printed upon circular discs of paper, the rising and falling temperatures being recorded by a line which moves at a greater or less distance from the centre, and those upon which the scale is approximately rectangular, with the rising and falling temperatures tracing a line which runs in the general direction of one edge of the paper. For most purposes the latter type of scale is preferable. Among the best of these instruments are those made by Jules Richard of Paris. Scales are printed in either centigrade or fahrenheit degrees. An instrument closely resembling that of Jules Richard is sold by Queen & Co. This type is suggested as a standard.

2. Humidity.

STANDARD METHOD FOR HUMIDITY. Although not always strictly accurate, especially at low temperatures, the most generally useful instrument for determining humidity is the psychrometer or wet and dry bulb thermometer. This instrument is made in several types, that employed by the United States Weather Bureau being simple, efficient and economical. The psychrometers employed by the Weather Bureau are of two principal kinds. In one case the two thermometers with their

wet and dry bulbs are whirled in a vertical plane by means of a small machine actuated by hand power. In the second, which is the most convenient for ordinary work, the thermometers are provided with a suitable handle by which the apparatus is whirled about by the hand of the investigator. The instrument suggested as standard is of the latter (1908) type modified slightly by Soper. It consists of two mercurial thermometers 24 centimeters long, graduated from —10 to 125 degrees Fahrenheit, fastened upon an aluminum back, 1.5 cm. apart center to center. The bulbs project beyond the aluminum back for 5 cm., one of the bulbs being covered with cloth. The upper end of the aluminum back is connected by two loose wire links with a substantial handle by which it can be whirled. The whole is carried in a cylindrical aluminum case. This instrument may be obtained from Schneider Bros., 265 Green St., N. Y., or from Queen & Co. The manner of use is fully described in Bulletin No. 235 of the U. S. Weather Bureau, which contains the full tables necessary for calculating humidity from the wet and dry bulb readings, and is also described in Ward's Meteorology (Ward, 1899).

Stationary wet and dry bulb thermometers mounted, as commonly seen, with a heavy backing are not suitable for the determination of relative humidity, owing to their lag and the likelihood that the wet bulb will not be suitably moistened or ventilated.

The hair hygrometer whose action depends upon the extension and contraction of a suitably prepared hair under the influence of moisture can be made an accurate instrument; and some types are arranged for continuous record. Certain forms of the instrument are open to the same objection which has been raised against thermometers which have a backing; there is difficulty in causing a sufficient current of air to come in contact with them.

3. Dust.

The simplest and one of the most useful methods of determining the amount of dust and its composition is by means of suitable receptacles, such as Petri dishes, upon which the dust is allowed to settle for a sufficient period of time to enable a considerable quantity to accumulate. Particles are then examined under a microscope, or, if desired, they can be swept by means of a camel's hair brush upon a watch glass and weighed.

It is a practicable and desirable procedure to filter air through cotton filters or filters of other material, the quantity of air being measured either by means of a gas meter or other device. Whatever the filtering medium the quantity of air should be large, in order that the quantity of dust may be appreciable in amount and fairly representative in quality. By weighing the filtering

material before and after passing the air through it the aggregate weight of dust in the quantity of air taken for examination can be determined. It is necessary, in most cases, to guard against increase in weight of the filtering material through the absorption of water. This can be done by placing the filtering material in a desiccator before and after filtration and just before weighing in each case.

STANDARD METHOD FOR DUST DETERMINATIONS IN ORDINARY AIR. For very careful work the number of dust particles in the atmosphere can be determined by an instrument invented by Professor John Aitken and called a dust counter. This instrument is expensive; and a somewhat smaller but more generally useful instrument, devised also by Aitken, and called the Koniscope is recommended for standard determinations. The dust counter and koniscope operate upon the principle that dust particles form nuclei upon which moisture condenses and precipitates from a saturated atmosphere. In the dust counter the droplets are counted, in the koniscope the opacity of cloud is estimated. There are not, apparently, many cases in which the dust counter can be turned to practical account in sanitary investigations.

The Koniscope consists of two brass tubes connected at right angles and suitably fitted with stopcocks and a small air pump. By exhausting the air from one of the tubes, allowing the space to become saturated with water vapor by evaporation from wet blotting paper within, and then allowing this moisture to condense upon the dusty astmophere under examination, clouds of different degrees of density can be formed inside the tube. The density of the clouds can approximately be measured by looking through the tube from one end to the other, windows being provided for this purpose. A table is supplied with the instrument to give the approximate number of dust particles corresponding to clouds of different degree of density.

The koniscope can be obtained from Queen & Co. This instrument is easily handled and sufficiently delicate to merit wider use than has yet been made of it in sanitary investigations. It is capable of detecting with great delicacy different currents of air, where the only difference between them lies in the number of dust particles present.

STANDARD METHOD FOR DUST DETERMINATIONS IN AIR HEAVILY LADEN WITH DUST PARTICLES. One of the principal objections to filtration methods in studying the dust in ordinary air lies in the fact that enormous volumes of air must be filtered in order to obtain appreciable results. In factories and other places where the dust is thick the following method is recommended. A measured volume of air is drawn through a filter of granulated sugar, and the sugar is dissolved and the dust suspended in a measured volume of distilled water. The volume taken must vary with the amount of dust present in the air. The sugar should be of the ordinary granulated type with grains between .25 and 1.00 mm. in diameter. The layer of sugar should be 1 cm. deep and may be held in place in a glass tube of 1 or 2 cm. bore by a perforated stopper and square of bolting cloth or by a plug of cotton. The air sample should be collected rather rapidly; for heavily laden air a suction cylinder of metal with a closely fitting piston may be used. Where larger volumes of air are to be examined a Roots blower, operating on the suction principle, can be used to advantage, the quantity of air being measured by a gas meter interposed between the blower and the filter.

The weight of dust present may be determined by filtering the water in which the dust has been suspended through a Gooch crucible. The number of dust particles may be found by the following method (Winslow, 1908): After thorough agitation, one c. c. of the suspension is placed in a Sedgwick-Rafter cell and the particles are counted under the microscope by the method used in the microscopical enumeration of micro-organisms in drinking water (Whipple, 1905). The cell is 50 mm. by 20 mm. in area and 1 mm. deep and the method employed consists essentially in counting the number of particles in representative mm. squares. Both the top and bottom of the cell must be examined to get dusts lighter and heavier than water. Glassware and sugar must be clean and control determinations should be made, to detect any chance pollution.

4. Illumination.

Two general methods are available for the practical determination of the intensity of lighting. The first of these methods depends upon the distance at which print of a given size can be read by an investigator possessing average eyesight. A card of type of different sizes such as is commonly employed by oculists is taken to the point where the light is to be measured and some line of type is selected for the test. The distance at which this type must be held from the eyes in order to be legible is then measured and compared with the distance at which the same type can be seen in unobstructed daylight. The difference between the two distances is taken as a basis of difference in the strength of the illumination.

Standard Method for Measuring Illumination. The second method, which is recommended as a standard procedure, depends on the use of photo-sensitive paper such as can be obtained from any dealer in photographic materials. By exposing the sensitized paper through a slot in a cardboard for a sufficient period of time and noting the number of seconds or minutes consumed to match in depth a standard shade of color the intensity of light can be determined with much accuracy. If a fresh piece of paper is exposed to the direct rays of the sun for three seconds it will assume a shade which can be used as a standard for a given series of tests. The intensity of light at other points may be compared with this by noting the number of seconds required to color a fresh piece of paper from the same lot to the same shade.

5. Velocity of Air Currents.

The velocity of strong air currents is customarily measured by means of recording anemometers. There are so many of these instruments on the market and their use is so generally understood that it seems unnecessary to describe them. Anemometers require a considerable velocity of air and they should never be used without a carefully prepared table of corrections whereby their readings can be adjusted.

It often becomes desirable in sanitary investigations, particularly in studies of ventilation, to determine the strength and direction of currents of air which are too delicate to be measured by means of anemometers. Lighted candles have sometimes been used to show the direction of such delicate air currents, the flame being deflected in the direction in which the current is moving. More delicate than this is the method of noting the course taken by the smoke from a joss stick, cigarette or cigar. For a further discussion of the study of air currents reference may be made to Shaw (1907).

6. Notes on Physical Determinations.

Physical observations of the atmosphere to be of value must not only be made with accuracy and with instruments suitable to the particular tests made, but the observations should be sufficiently numerous to indicate representative or, at least, average conditions at the place under inquiry. One determination of temperature or humidity, for example, is of little service unless it is known that the circumstances under which that determination was made frequently occur. No instrument, of course, is mathematically exact. Each has its error and it is important to learn its error and allow for it whenever failure to do so would affect the value of the results desired. The difficulties of adjustment and uncertainty of results obtained with very delicate apparatus in the hands of unskilled workers make the recommendation of the most refined instruments seem unwise in this place, where practical rather than ultra-scientific methods are desirable.

It is important in using any of the physical instruments referred to here that their accuracy be not over-rated. All instruments employed in sanitary investigations should be thoroughly understood by the investigator using them, and where any considerable importance attaches to the results the instruments should be standardized. To standardize an instrument is to compare it with some other instrument whose accuracy has been demonstrated and its error known. An extensive investigation should be carried on by the help of a special testing station, where all the instruments can be standardized and examined from time to time by a person especially assigned to this work.

In the absence of a testing station or other convenient means of standardizing instruments, apparatus for the physical examination of air can be sent to the Bureau of Standards, Washington, D. C. At that Bureau examinations can be made of thermometers and other instruments and the results reported upon at a nominal cost. Every laboratory and sanitary worker should have a few instruments which have been tested by this or some other laboratory and can be depended upon as accurate enough to be used for comparison.

III. Chemical Determinations.

1. Laboratory Methods for Determining Carbon Dioxide with a High Degree of Accuracy.

Numerous efforts have been made to develop methods of analyzing air for carbon dioxide, applicable to the varying conditions under which the chemist, sanitary engineer or inspector must work. The chemist is called upon to make exceedingly accurate, careful analyses for scientific purposes, while the inspector and engineer are called upon to make estimates and comparisons. It is plain that no one system or method will satisfactorily meet the requirements of all these conditions and therefore in preparing a description of the most satisfactory processes for use as standard methods, the available methods have been classed either as accurate methods or as general tests.

For accurate, scientific work, say, when accuracy to 1-10 of a part per ten thousand is required, the committee recommends as the standard the Patterson apparatus as modified by Sondén, one form of which has been used by Dr. F. G. Benedict of the Carnegie Nutrition Laboratory for over a year, with the greatest satisfaction.*

This apparatus measures a given volume of air, and absorbs the contained carbon dioxide in potassium hydroxide, afterward accurately measuring the remainder, thus giving the carbon dioxide present by volume. The air is measured in all cases at the same pressure and temperature and is measured accurately by means of the readings on a very finely graduated capillary. The principle is simple, but accurate operation requires considerable technique.

* This apparatus will shortly be described in print by Dr. Benedict.

The apparatus may be had by applying to Sondén in Stockholm at a cost of something less than one hundred dollars.

For accurate inspection work, say, one-quarter of a part per ten thousand, the Eimer and Amend form of the Petterson Palmquist apparatus is recommended. This is very similar to the Sondén form but not as delicate. Its cost is about fifty-five dollars.

2. Practical Methods of Determining Carbon Dioxide for Sanitary Purposes.

The time method of Cohen and Appleyard (1894), is recommended as combining practicability and reasonable accuracy in a degree suitable for practical sanitary work.

STANDARD METHOD FOR CARBON DIOXIDE. If a dilute solution of lime water, slightly colored with phenolphthalein, is brought in contact with air containing more than enough CO_2 to combine with all the lime present, the solution will be gradually decolorized, the length of time required depending upon the amount of CO_2 present. The quantity of lime water and volume of air remaining the same, the rate of decolorization varies inversely with amount of carbon dioxide. The method is scientific in principle because it recognizes the fact that the absorption of CO_2 by Ca or Ba hydroxide solution is a time reaction.

Collect samples of air in one-half liter glass-stoppered bottles by any of the methods of collection. Run in 10 cc. standard lime water, replace stopper, and note time. Shake bottle vigorously with both hands until color disappears. Note time required, and ascertain corresponding amount of CO_2 from table.

TABLE.

Time in Minutes to Decolorize Solution	CO_2 per 10,000	Time in Minutes to Decolorize Solution	CO_2 per 10,000
1¼	16.0	3½	6.0
1⅓	13.8	4	5.3
1½	12.8	4¼	5.1
2	12.0	5	4.6
2¼	11.5	5¼	4.4
2¾	8.6	6¼	4.2
3¼	7.7	7½	3.5

3. Rough Methods of Determining Carbon Dioxide.

For the sake of completeness a brief description of the shaker methods of determining carbon dioxide is here included, although their accuracy is not such as to warrant the committee in recommending their use.

The volume of air that must be brought into contact with a definite quantity of lime water, in order to neutralize all the lime, is taken as a measure of the CO_2 in the air. The quantity of lime water and the time of reaction remaining constant, the amount of CO_2 varies inversely as the volume of air. The apparatus consists of graduated shakers either Wolpert or Fitz, and a pipette for measuring 10 c. c. of lime water.

Be sure the plunger of the shaker slides easily, then remove and run into the tube 10 c. c. of the lime water solution. Introduce the plunger, and press it to the top of the solution, then withdraw it to the higher graduation. Close the mouth of the small tube in the Fitz, or the stem of the plunger in the Wolpert with the finger and shake vigorously for 30 seconds. The volume of air brought in contact with the solution is 50 c. c. in the Fitz and 40 c. c. in the Wolpert. Remove finger closing small end, press inner tube or plunger again to top of solution in Wolpert or to T in Fitz, and draw it up as before, thus admitting 20 c. c. fresh air in the Fitz and 40 in the Wolpert. Shake for 30 seconds. Repeat until color is discharged. The first trial will probably give the approximate result, and subsequent tests will aid in giving the correct one. From the volume of air used, the amount of CO_2 can be determined from the table.

TABLE.

Air in cc. Used	CO_2 per 10,000	Air in cc. Used	CO_2 per 10,000
30	28	91	9 bad
36	22	103	8
46	18 very bad	117	7
58	14	138	6
69	12	165	5 good
82	10	207	4

Stoppers and vials should be washed and dried and kept separate and parts of the shaker should be kept separate. In using the shaker see that the fingers are clean. Take care to avoid loss of liquid on addition of fresh air.

4. Methods of Collection.

In the case of the Cohen and Appleyard Method.

Fully as important as the actual test is the method of collecting the sample. For this the committee recommends as standard for more accurate work, the method of collection by water siphon.

STANDARD METHOD OF COLLECTION. The Water Siphon Method. Two bottles (diameter one-third the height), volume about one-half litre, of nearly equal capacity should be fitted with rubber stoppers carrying small glass tubing connected by several feet of rubber connector, with clamps. Fill one bottle completely with water, nearly free from carbon dioxide.

The pair of bottles is taken to the place from which the air is to be collected. The inlet tube may be long to reach to near the ceiling, or short; if long, the first siphoning should be rejected, to secure filling the inlet tube with the air desired, the stoppers exchanged, and the sample taken. The air-filled bottle should be stoppered and taken to the laboratory; or the test solution at once added, and the bottle stoppered and shaken, noting minutes and seconds. One bottle of water with a small reserve will serve for a number of takings before absorbing a deleterious amount of CO_2.

The Steam Vacuum Method may be used as an alternative in less accurate work. The steam is supplied by a 500 c. c. flask serving as a boiler, with a bunsen burner to apply the heat. The flask should be fitted with a rubber stopper carrying a No. 6 glass tube so arranged that one end extends within ½ inch of the bottom of the bottle when placed in position on the stand. The bottles should be of about 500 c. c. capacity, made for a ground-glass stopper but fitted with a rubber stopper.

To prepare the jet, the water in the flask should boil for five minutes in order to expel completely the air in the water and the flask. The pressure should be sufficient to throw the vaporized steam at least 1 foot above the exposed end of the tube.

Place the empty bottle on the stand in an inverted position and allow to remain for three minutes. In the meantime apply a thin coating of vaseline half way up the sides of the stopper. The vaseline acts as an unguent, reducing the coefficient of friction to such an extent that the principal resistance is due to the reaction of the stopper against compression. This enables one

to force the stopper in far enough to bring the glass and rubber into intimate contact, which is essential. The vaseline also fills the interstices between the rubber and the glass, so as to make leakage impossible.

Protecting the hand with a cloth, raise the bottle from the stand, and the instant it clears the end of the tube insert the stopper while the bottle is still inverted. The stopper may be pushed in more securely by pushing it against the table with a few pounds pressure while the bottle is still in the inverted position. Keep the stopper in under this pressure for a few minutes until the vacuum begins to form, after which the atmospheric pressure will keep it in place.

All the bottles required are treated in the same way. The rubber stopper should be at least one size larger than would ordinarily be used for the bottle, and should project three-eighths of an inch or more so as to be easily removed when the sample is to be taken.

Sample bottles may be tested for completeness of vacuum by holding them in an inverted position under water at 70° F., and removing the stopper. After the water has replaced the vacuum, the stopper is inserted and the bottle removed.

5. Solutions.

STANDARD LIME WATER FOR GENERAL TESTS. To a litre of distilled water add 2.5 cc. of phenolphthalein (made by dissolving .7 grams of phenolphthalein in 50 cc. of alcohol and adding an equal volume of water). Stand the bottle of water on a piece of white paper and add drop by drop saturated lime water till a faint color persists for a full minute. Now add 6.3 cc. of saturated lime water and quickly cork the bottle, or connect the pipette.

IV. BACTERIOLOGICAL DETERMINATIONS.

The determination of the number of bacteria in air seems to the Committee to have less importance than was once believed. Disease spread through air is probably due most often to direct pollution with spray from the mouth; and it does not seem possible to measure such pollution in a quantitative way. The total number of saprophytic bacteria often corresponds with the amount of dust present. This is especially true when the dust is not of metallic or other industrial origin. In the examination of the air of barns, dairies, theatres, factories and streets bacterial data may prove of value.

1. Quantitative Determinations: A large number of different pieces of apparatus have been devised which are, after all, simply adaptations of three general methods, viz.:

(a) Filtration of air; (b) Bubbling air through some liquid medium; (c) Precipitating the bacteria from a given volume of air. While each of these methods can be made to give fairly satisfactory quantitative data in the hands of competent workers, nevertheless the committee is of the opinion that the time has arrived when one of them should be adopted as a standard and the others preferably dropped. In adopting a method as standard, the following principles should govern the selection:

(a) Simplicity and inexpensiveness of apparatus.
(b) Ease of operation.
(c) Universal applicability.

Basing judgment upon these considerations and upon numerous comparative tests made for the purpose, (Weinzirl and Fos), the Committee is of the opinion that the filtration method comes nearer to the ideal than either of the other two, and, therefore, that it should be adopted as standard. The apparatus and procedure is described as follows:

STANDARD METHOD FOR ENUMERATING BACTERIA IN AIR. (Filtration method of Petri). The filter tubes are glass tubes 1½ cm. in diameter and 10 cm. long. In the end of each is placed a perforated cork stopper through which a glass tube 6 mm. in diameter is passed. The filter consists of a layer of sand which has been passed through a 100 mesh sieve, 1 cm. deep supported by a layer of bolting cloth covering the cork. Two filter tubes are connected in tandem and a measured volume of air, 10 litres or more, is drawn through at a constant rate by suction. The suction is applied by means of an aspirator of known volume, preferably one of the double or continuous type. Either the Aspirator, Magnus (No. 12,210, $7.50–$9.00), or the Double Aspirator (No. 12,212, $20–$25), both made by Bausch and Lomb are suitable for this purpose. Before using a pair of filter tubes, a test for possible leakage is made by placing the thumb over the cotton stopper and applying the aspirator; if the suction is weak or absent, the corks must be tightened or the tubes discarded. All corks should be tightened and connections wired immediately before using the filters. The collection of the sample should take from 1 to 2 minutes, per liter.

After filtering a definite volume of air through the tubes, the sand is shaken out into 10 cc. of sterile water, thoroughly shaken and aliquot portions plated in ordinary nutrient agar, all plates being made in duplicate. The plates are incubated at room temperature for five days, when final counts are made. If petri dishes 9 cm. in diameter are employed, all plates showing a larger count than 200 colonies should be rejected to eliminate inhibitive action.

A rough idea of the bacterial content of the air may be obtained by the method of exposing plates for definite periods of time and counting the colonies which develop from the germs falling upon them. This procedure is not, however, recommended by the Committee on account of the fact that results are notably affected by varying environmental conditions and are not related to any specific volume of air.

2. Qualitative Determinations: In the study of the bacteriology of sewer air the colon bacillus and the sewage streptococcus may conveniently be used as indices of contamination. Samples may be collected as for the quantitative determination and, after incubating the filtering sand in dextrose broth, must be kept for at least a week and examined daily after three days. Any streptococcus producing a faint growth on agar and coagulating milk may be considered as of human origin.

Gordon (1904) has suggested the use of the similar streptococci found in the mouth as indices of pollution by mouth spray. Your committee (Winslow and Robinson, 1909) has been unable to find such organisms in the air, even under extreme conditions, in sufficient numbers to warrant the recommendation of this test.

C.-E. A. Winslow, Chairman.
Ellen H. Richards,
G. A. Soper,
J. Bosley Thomas,
John Weinzirl.

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